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L6: Entry 20 of 33

File: USPT

Jun 17, 1997

DOCUMENT-IDENTIFIER: US 5639603 A

TITLE: Synthesizing and screening molecular diversity

DEPR:

Parallel synthesis strategies require the use of a set of protecting groups on the amino acids and nucleotide building blocks that are mutually orthogonal, and that each of the polymer chains be stable to the reagents used in the synthesis and deprotection of the second chain. Although, in principle, a variety of protection/deprotection schemes can be used (as discussed above), Fmoc/.sup.t Bu protection on the peptide building blocks is preferred, because of the extensive commercial availability of natural and unnatural amino acids protected in this manner. However, the .sup.t Bu-based peptide side chain protecting groups require treatment with strong acid (typically trifluoroacetic acid) for removal, conditions that can lead to rapid depurination of oligonucleotides containing either deoxyadenosine (dA) or 2'-deoxyguanosine (dG) (see Capon, 1969, Chem. Rev. 69: 407-498, incorporated herein by reference). This problem has been circumvented by using 7-deaza-2'-deoxyadenosine (c.sup.7 dA) in place of dA in the template oligonucleotide tag. The glycosidic bonds of deazapurine nucleosides are resistant to acid-catalyzed hydrolysis (see Scheit, 1980, Nucleotide Analogs: Synthesis and Biological Function (John Wiley and Sons, New York) pp. 64-65, incorporated herein by reference), and oligonucleotides incorporating these monomers are faithfully copied by thermostable polymerases used in the PCR (see McConlogue et al., 1988, Nucl. Acids Res. 16: 9869, and Barr et al., 1986, BioTechniques 4: 428-432, each of which is incorporated herein by reference). Acid-resistant guanosine analogs can also be incorporated into the template DNA.

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L6: Entry 2 of 33

File: USPT

Jul 18, 2000

DOCUMENT-IDENTIFIER: US 6090590 A

TITLE: Reducing nontemplated 3' nucleotide addition to polynucleotide transcripts

BSPR:

The heterogeneity at the 3' ends of transcripts generated by polymerases in vitro can have detrimental effects in a number of applications. In the production of infectious viral transcripts, 3' heterogeneity may reduce the synthesis by viral RNA replicase (Sun et al., 1996, Virology 226:1-12.), or reduce the infectivity of the RNA (Murphy & Park, 1997, Virology 232: 145-157). In biochemical and structural analyses of RNA structures, 3' heterogeneity may result in additional signals that complicate the interpretation of results and may prevent the effective crystallization of the RNA needed for X-ray crystallography (Price et al., 1995, J. Mol. Biol. 249: 398-408.). Previously, one commonly used strategy to generate a homogeneous RNA sample of less than 50-nts is to perform denaturing gel electrophoresis and to excise and elute the desired band (Wyatt et al., 1991, BioTechniques 11:764-769.). For RNAs longer than 50-nts, where it is difficult to separate the N+1 RNA from the desired RNA by gel electrophoresis, the transcript can be expressed with a cis-acting ribozyme sequence that will cleave at the desired positions (Price et al., 1995, supra), or by use of a mutant T7 RNA polymerase that is defective in its initiation rate (Gardener et al., 1997, Biochemistry 36:2908-18.). Alternatively, Moran et al., (1996, Nucl. Acids Res. 24: 2044-2052) demonstrated that DNA templates containing nucleoside with base analogs that cannot form hydrogen bonds with the substrate nucleotide can induce the termination of transcription prior to the nucleoside analog. However, these analogs are not widely available, may alter transcriptional fidelity, and often leave a significant amount of N+1 activity.

BSPR:

In studies of RNA synthesis by a viral RNA-dependent RNA polymerase, we noticed that nontemplated nucleotide addition on the nascent RNA was decreased when the 5' terminus of the template was modified with deoxyribonucleotides (Siegel et al., 1999, J Virol 73(8):6424-6429). We subsequently determined that a DNA template containing nucleotide analogs at the 5' terminus would similarly affect RNA synthesis by the T7 RNA polymerase and have now extended these findings to a wide range of templates, transcripts and polymerases. Accordingly, we show how non-template 3' nucleotide addition to a transcript can be reduced by employing a template comprising an ultimate and/or penultimate 5' ribose having a terminator substituent at the C'2 position.

DEPR:

Transcription from DNA template containing ribose 2'-methoxy analogs. As an initial test, we used a DNA named S5 which should encode a 22-nt RNA within P5 of the Tetrahymena thermophila group I intron. For nomenclature,

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L2: Entry 4 of 5

File: USPT

Mar 28, 2000

DOCUMENT-IDENTIFIER: US 6043031 A

TITLE: DNA diagnostics based on mass spectrometry

DEPR:

For the 99-mer primers 1 and 2, for the 200-mer primers 1 and 3 and for the 100-mer primers 6 and 7 were used. To obtain 7-deazapurine modified nucleic acids, during PCR-amplification dATP and dGTP were replaced with 7-deaza-dATP and 7-deaza-dGTP. The reaction was performed in a thermal cycler (OmniGene, MWG-Biotech, Ebersberg, Germany) using the cycle: denaturation at 95.degree. C. for 1 min., annealing at 51.degree. C. for 1 min. and extension at 72.degree. C. for 1 min. For all PCRs the number of reaction cycles was 30. The reaction was allowed to extend for additional 10 min. at 72.degree. C. after the last cycle.

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L2: Entry 5 of 5

File: USPT

Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843669 A

TITLE: Cleavage of nucleic acid using thermostable methanococcus jannaschii FEN-1 endonucleases

DEPR:

The term "nucleotide analog" as used herein refers to modified or non-naturally occurring nucleotides such as 7-deaza purines (i.e., 7-deaza-dATP and 7-deaza-dGTP). Nucleotide analogs include base analogs and comprise modified forms of deoxyribonucleotides as well as ribonucleotides. As used herein the term "nucleotide analog" when used in reference to substrates present in a PCR mixture refers to the use of nucleotides other than dATP, dGTP, dCTP and dTTP; thus, the use of dUTP (a naturally occurring dNTP) in a PCR would comprise the use of a nucleotide analog in the PCR. A PCR product generated using dUTP, 7-deaza-dATP, 7-deaza-dGTP or any other nucleotide analog in the reaction mixture is said to contain nucleotide analogs.

DEPR:

The effect of using various nucleotide analogs to generate substrates for CFLP.RTM. reactions was examined. As discussed below, nucleotide analogs are used in PCRs for several reasons; therefore, the ability to analyze the modified products of PCRs (i.e., nucleotide analog-containing PCR products) by CFLP.RTM. analysis was investigated. The 7-deaza purine analogs (7-deaza-dATP and 7-deaza-dGTP) serve to destabilize regions of secondary structure by weakening the intrastrand stacking of multiple adjacent purines. This effect can allow amplification of nucleic acids that, with the use of natural dNTPs, are resistant to amplification because of strong secondary structure [McConlogue et al., Nucleic Acids Res. 16:20 (1988)].

DEPR:

For these comparisons, substrates corresponding to a 157 bp fragment derived from exon of the wild-type and R422Q mutant of the human tryosinase gene were generated by PCR amplification using either 1) the standard mixture of dNTPs (i.e., dATP, dCTP, dGTP and dTTP); 2) dUTP in place of dTTP; 3) 7-deaza-dGTP (d.sup.7 GTP) in place of dGTP; and 4) 7-deaza-dATP (d.sup.7 ATP) in place of dATP. These substrates were then incubated with Cleavase.RTM. BN enzyme and the effect the presence of the various nucleotide analogs on the cleavage pattern was examined.

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